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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

YUJI HATADA ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: 09/985,689 :

FILED: November 5, 2001 :

FOR: ALKALINE PROTEASES :

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Responsive to the Notice dated January 16, 2002, Applicants submit herewith an Amendment, a substitute Abstract of the Disclosure, a substitute Sequence Listing, and a corresponding computer-readable Sequence Listing. Prior to examination on the merits, please amend the above-identified application as follows.

IN THE ABSTRACT OF THE DISCLOSURE

Please delete the original Abstract of the Disclosure at pages 62 to page 63 and replace with the substitute Abstract of the Disclosure attached hereto.

IN THE SPECIFICATION

Please replace the paragraph bridging pages 18 and 19 with the following:

--To the detergent composition of the present invention, various enzymes can be used in combination with the alkaline protease of the present invention. Examples include hydrolases, oxidases, reductases, transferases, lyases, isomerases, ligases and synthetases. Of these, proteases, cellulases, lipases, keratinases, esterases, cutinases, amylases, pullulanases, pectinases, mannases, glucosidases, glucanases, cholesterol oxidases, peroxidases, laccases and proteases other than the alkaline protease used in the present invention are preferred.--

Please replace the paragraph bridging pages 21 and 22 with the following:

--Mutation was introduced at random into a protease structural gene of about 2.0 kb including a termination codon by the following manner. First, PCR was conducted using a primer capable of amplifying this 2.0kb. A PCR master mix contained 5 ng of a template DNA, 20 pmoL of a phosphorylated primer, 20 nmoL of each dNTP, 1 μmoL of Tris/HCl (pH 8.3), 5 μmoL of KCl, 0.15 μmoL of $MgCl_2$ and 2.5U TaqDNA polymerase, and its total amount was adjusted to 100 μL. After modification of the template by allowing it to stand at 94°C for 5 minutes, PCR was performed for 30 cycles, each cycle consisting of treatment at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1.5 min. The PCR product was purified by "PCR product purification Kit" (product of Boeringer Manheim), followed by elution in 100 μL of sterile water. With 1 μL of the eluate, second PCR was conducted under conditions similar to those of the first PCR except for the template DNA. After completion of the second PCR, the PCR product was purified in a similar manner to the first PCR, followed by elution in 100 μL of sterile water.--

Please replace the paragraph bridging pages 22 and 23 with the following:

--The amplified DNA fragment was integrated in a vector by polymerase reaction using "LATaq" produced by Takara Shuzo Co., Ltd. Described specifically, after addition of

5 μL of a buffer for LATaq (a 10-fold concentrate), 8 μL of a dNTP solution and 0.5 μL of LATaq DNA polymerase, and as a template, 20 ng of plasmid pHA64TS (having a protease structural gene linked with an expression vector pHA64) to 35 μL of the purified eluate, the total amount was adjusted to 50 μL. PCR reaction of the resulting liquid was carried out for 30 cycles, each consisting of treatment at 94°C for 1 min, 55°C for 1 min and 72°C for 4 min. By the subsequent ethanol precipitation, the PCR product was collected. This PCR product had a shape of a plasmid having a nick at the 5' prime end of the primer. Ligase reaction by T4 ligase (product of Takara Shuzo Co., Ltd.) was conducted to link this nick portion.--

Page 23, please replace the paragraph beginning at line 11 with the following:

--By using 10 μL of this ligase reaction mixture, transformation of the *Bacillus subtilis* strain ISW1214 was conduced, whereby about 4×10^5 transformants were obtained. The resulting transformants of the strain ISW1214 were cultured on a skin-milk-containing medium (containing 1% skim milk, 1% bactotrypton, 1% sodium chloride, 0.5% yeast extract, 1.5% agar and 7.5 μg/ml of tetracycline) and halo formation, which was presumed to reflect the protease secretion amount, was observed.--

Please replace the paragraph bridging pages 23, 24 and 25 with the following:

--The protease active fraction was prepared in the following manner. The transformants obtained in Example 1 was cultured at 30°C for 60 hours on a medium A (3% polypeptone S (product of Nippon Pharmaceutical), 0.5% yeast extract, 1% fish meat extract (product of Wako Pure Chemical Industries, Ltd.), 0.15% dipotassium phosphate, 0.02% magnesium sulfate 7 hydrate, 4% maltose and 7.5 μg/mL of tetracycline). The supernatant of the thus-obtained cultured medium was added with ammonium sulfate to give 90%

saturation, whereby salting-out of protein was caused. The sample obtained by salting-out was dissolved in a 10 mM tris HCl buffer (pH 7.5) containing 2 mM of calcium chloride. The resulting solution was dialyzed overnight against the same buffer by using a dialysis membrane. The fraction in the dialysis membrane was applied to DEAE Bio-Gel A (product of Bio-Rad Laboratories) equilibrated with a 10 mM tris HCl buffer (pH 7.5) containing 2 mM calcium chloride to collect the protease active fraction not adsorbed to the ion-exchanger. This active fraction was applied further to "SP-Toyopearl 550W" (product of Tosoh Corp.) equilibrated with the same buffer, followed by elution with a 0 to 50 mM sodium chloride solution, whereby a protease active fraction was obtained. The resulting fraction was analyzed by SDS-PAGE electrophoresis to confirm that the protease was obtained as substantially uniform protein. The protein concentration was measured in accordance with the method of Lowry, et al. (J. Biol. Chem. **193**, 265-275(1981)) by using bovine serum albumin (product of Bio-Rad Laboratories) as a standard.--

Please replace the paragraph bridging pages 30 and 31 with the following:

--In 2 mL of a 100 mM borate buffer (pH 10.5) containing 3% of aqueous hydrogen peroxide, a 50 μL portion of each of the protease variants obtained by purification in Example 1 was added. The resulting mixture was allowed to stand at 30°C for 30 minutes. After addition of an adequate amount of catalase (product of Boehringer Manheim) to remove excess hydrogen peroxide, the residual protease activity was measured by the synthetic substrate assay. In FIG. 3, the residual activity after treatment with aqueous hydrogen peroxide is shown relative to the activity before treatment set at 100%.--

Please delete the original Sequence Listing from page 32 to page 56 of the specification.

Page 63 (Abstract), after the last line, beginning on the next page, insert the substitute Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as follows:

--1. (Amended) An alkaline protease, wherein an amino acid residue at (a) position 84, (b) position 104, (c) position 256 or (d) position 369 of SEQ ID NO:1 or at a position corresponding thereto has been deleted or selected from:

at position (a): an arginine residue,

at position (b): a proline residue,

at position (c): an alanine, serine, glutamine, valine, leucine, asparagine, glutamic acid or aspartic acid residue, and

at position (d): an aspartic acid residue

2. (Amended) An alkaline protease, comprising an amino acid sequence represented by SEQ ID NO:1 or having an amino acid sequence showing at least 60% homology therewith, wherein an amino acid residue at (a) position 84, (b) position 104, (c) position 256 or (d) position 369 of SEQ ID NO:1 or at a position corresponding thereto has been deleted or selected from:

at position (a): an arginine residue,

at position (b): a proline residue,

at position (c): an alanine, serine, glutamine, valine, leucine, asparagine, glutamic acid or aspartic acid residue, and

at position (d): an aspartic acid residue.

3. (Amended) An alkaline protease, wherein an amino acid residue at (e) position 66 or 264, (f) position 57, each of 101 to 106, 136, 193 or 342, (g) position 46 or 205, (h)

position 54, 119, 138, 148 or 195, (i) position 247, (j) position 124, (k) position 107 or (l) position 257 of SEQ ID NO:1, or at a position corresponding thereto has been deleted or selected from:

at position (e): a glutamine, aspartic acid, serine, glutamic acid, alanine, threonine, leucine, methionine, cysteine, valine, glycine or isoleucine residue

at position (f): a lysine, serine, glutamine, phenylalanine, valine, arginine, tyrosine, leucine, isoleucine, threonine, methionine, cysteine, tryptophan, aspartic acid, glutamic acid, histidine, proline or alanine residue,

at position (g): a tyrosine, tryptophan, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, lysine, glutamine, methionine or cysteine residue,

at position (h): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, histidine, serine, lysine, glutamine, methionine, glycine, aspartic acid, proline, arginine or cysteine residue,

at position (i): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, glutamine, methionine or cysteine residue,

at position (j): an alanine or lysine residue,

at position (k): a lysine, arginine, alanine or serine residue, and

at position (l): a valine or isoleucine residue.

4. (Amended) An alkaline protease, comprising an amino acid sequence represented by SEQ ID NO:1 or having an amino acid sequence showing at least 60% homology therewith, wherein an amino acid residue at (e) position 66 or 264, (f) position 57, each of 101 to 106, 136, 193 or 342, (g) position 46 or 205, (h) position 54, 119, 138, 148 or 195, (i)

position 247, (j) position 124, (k) position 107 or (l) position 257 has been deleted or selected from:

at position (e): a glutamine, aspartic acid, serine, glutamic acid, alanine, threonine, leucine, methionine, cysteine, valine, glycine or isoleucine residue

at position (f): a lysine, serine, glutamine, phenylalanine, valine, arginine, tyrosine, leucine, isoleucine, threonine, methionine, cysteine, tryptophan, aspartic acid, glutamic acid, histidine, proline or alanine residue,

at position (g): a tyrosine, tryptophan, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, lysine, glutamine, methionine or cysteine residue,

at position (h): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, histidine, serine, lysine, glutamine, methionine, glycine, aspartic acid, proline, arginine or cysteine residue,

at position (i): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, glutamine, methionine or cysteine residue,

at position (j): an alanine or lysine residue,

at position (k): a lysine, arginine, alanine or serine residue, and

at position (l): a valine or isoleucine residue.

5. (Amended) The alkaline protease according to Claim 2, wherein the amino acid sequence represented by SEQ ID NO:1 or amino acid sequence showing at least 60% homology therewith is an amino acid sequence selected from a group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

6. (Amended) A gene encoding the alkaline protease according to Claim 1.

7. (Amended) A recombinant vector comprising the gene according to Claim 6.

8. (Amended) A transformant comprising the recombinant vector according to Claim 7.

9. (Amended) The transformant according to Claim 8, wherein a microorganism is used as a host.

10. (Amended) A detergent [composition] composition, comprising the alkaline protease according to Claim 1.--

Please add the following:

--11. (New) A gene encoding the alkaline protease according to Claim 2.

12. (New) A recombinant vector comprising the gene according to Claim 11.

13. (New) A transformant comprising the recombinant vector according to Claim 12.

14. (New) The transformant according to Claim 13, wherein a microorganism is used as a host.

15. (New) A detergent composition comprising the alkaline protease according to Claim 2.

16. (New) A gene encoding the alkaline protease according to Claim 3.

17. (New) A recombinant vector comprising the gene according to Claim 16.

18. (New) A transformant comprising the recombinant vector according to Claim 17.

19. (Amended) The transformant according to Claim 18, wherein a microorganism is used as a host.

20. (New) A detergent composition comprising the alkaline protease according to Claim 3.

21. (New) The alkaline protease according to Claim 4, wherein the amino acid sequence represented by SEQ ID NO:1 or amino acid sequence showing at least 60% homology therewith is an amino acid sequence selected from a group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

22. (New) A gene encoding the alkaline protease according to Claim 21.

23. (New) A recombinant vector comprising the gene according to Claim 22.

24. (New) A transformant comprising the recombinant vector according to Claim 23.

25. (New) The transformant according to Claim 24, wherein a microorganism is used as a host.

26. (New) A detergent composition comprising the alkaline protease according to Claim 21.

27. (New) A gene encoding the alkaline protease according to Claim 4.

28. (New) A recombinant vector comprising the gene according to Claim 27.

29. (New) A transformant comprising the recombinant vector according to Claim 28.

30. (New) The transformant according to Claim 29, wherein a microorganism is used as a host.

31. (New) A detergent composition comprising the alkaline protease according to Claim 4.

32. (New) A gene encoding the alkaline protease according to Claim 5.

33. (New) A recombinant vector comprising the gene according to Claim 32.

34. (New) A transformant comprising the recombinant vector according to Claim 33.

35. (New) The transformant according to Claim 34, wherein a microorganism is used as a host.

36. (New) A detergent composition comprising the alkaline protease according to Claim 5.

REMARKS

Claims 1-36 are pending.

The original Abstract of the Disclosure has been replaced by a substitute Abstract of the Disclosure in order to comply with proper form. No new matter is believed to be introduced by this amendment.

The specification has been amended to correct typographical errors. For example, the units "iL" have been changed to "μL". No new matter is believed to be introduced by these amendments to the specification.

The claims have been amended in order to place them in proper form and to eliminate multiple dependencies. Further, Claims 11-36 are new. Support for the new claims is found at pages 1-31 of the specification and the original claims. No new matter is believed to be introduced by the amendment to the claims and the addition of new claims.

Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing, and an amendment. Contents of the paper copy of the substitute Sequence Listing and the computer-readable Sequence Listing are identical.

Support for all the sequences listed in the substitute Sequence Listing can be found in the present application. No new matter is introduced by the submission of the substitute Sequence Listing and the computer-readable Sequence Listing.

Applicants submit that this application is now in condition for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Marked-Up Copy
Serial No: 09/985,689
Amendment Filed on:
HEREWITH

IN THE ABSTRACT OF THE DISCLOSURE

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IN THE SPECIFICATION

Please replace the paragraph bridging pages 18 and 19 with the following:

--To the detergent composition of the present invention, various enzymes can be used in combination with the alkaline protease of the present invention. Examples include hydrolases, oxidases, reductases, transferases, lyases, isomerases, ligases and synthetases. Of these, proteases, cellulases, lipases, keratinases, esterases, cutinases, amylases, pullulanases, pectinases, mannases, glucosidases, glucanases, cholesterol oxidases, peroxidases, laccases and proteases other than the alkaline protease used in the present invention are preferred.--

Please replace the paragraph bridging pages 21 and 22 with the following:

--Mutation was introduced at random into a protease structural gene of about 2.0 kb including a termination codon by the following manner. First, PCR was conducted using a primer capable of amplifying this 2.0kb. A PCR master mix contained 5 ng of a template DNA, 20 pmoL of a phosphorylated primer, 20 nmoL of each dNTP, 1 [imol] μmoL of Tris/HCl (pH 8.3), 5 [imol] μmoL of KCl, 0.15 [imol] μmoL of $MgCl_2$ and 2.5U TaqDNA

polymerase, and its total amount was adjusted to 100 [imol] μL. After modification of the template by allowing it to stand at 94°C for 5 minutes, PCR was performed for 30 cycles, each cycle consisting of treatment at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1.5 min. The PCR product was purified by "PCR product purification Kit" (product of Boeringer Manheim), followed by elution in 100 [iL] μL of sterile water. With 1 [iL] μL of the eluate, second PCR was conducted under conditions similar to those of the first PCR except for the template DNA. After completion of the second PCR, the PCR product was purified in a similar manner to the first PCR, followed by elution in 100 [iL] μL of sterile water.--

Please replace the paragraph bridging pages 22 and 23 with the following:

--The amplified DNA fragment was integrated in a vector by polymerase reaction using "LATaq" produced by Takara Shuzo Co., Ltd. Described specifically, after addition of 5 [iL] μL of a buffer for LATaq (a 10-fold concentrate), 8 [iL] μL of a dNTP solution and 0.5 [iL] μL of LATaq DNA polymerase, and as a template, 20 ng of plasmid pHA64TS (having a protease structural gene linked with an expression vector pHA64) to 35 [iL] μL of the purified eluate, the total amount was adjusted to 50 [iL] μL. PCR reaction of the resulting liquid was carried out for 30 cycles, each consisting of treatment at 94°C for 1 min, 55°C for 1 min and 72°C for 4 min. By the subsequent ethanol precipitation, the PCR product was collected. This PCR product had a shape of a plasmid having a nick at the 5' prime end of the primer. Ligase reaction by T4 ligase (product of Takara Shuzo Co., Ltd.) was conducted to link this nick portion.--

Page 23, please replace the paragraph beginning at line 11 with the following:

--By using 10 [iL] μL of this ligase reaction mixture, transformation of the *Bacillus subtilis* strain ISW1214 was conduced, whereby about 4×10^5 transformants were obtained.

The resulting transformants of the strain ISW1214 were cultured on a skin-milk-containing medium (containing 1% skim milk, 1% bactotrypton, 1% sodium chloride, 0.5% yeast extract, 1.5% agar and 7.5 [ig] μg/ml of tetracycline) and halo formation, which was presumed to reflect the protease secretion amount, was observed.--

Please replace the paragraph bridging pages 23, 24 and 25 with the following:

--The protease active fraction was prepared in the following manner. The transformants obtained in Example 1 was cultured at 30°C for 60 hours on a medium A (3% polypeptone S (product of Nippon Pharmaceutical), 0.5% yeast extract, 1% fish meat extract (product of Wako Pure Chemical Industries, Ltd.), 0.15% dipotassium phosphate, 0.02% magnesium sulfate 7 hydrate, 4% maltose and 7.5 [ig] μg/mL of tetracycline). The supernatant of the thus-obtained cultured medium was added with ammonium sulfate to give 90% saturation, whereby salting-out of protein was caused. The sample obtained by salting-out was dissolved in a 10 mM tris HCl buffer (pH 7.5) containing 2 mM of calcium chloride. The resulting solution was dialyzed overnight against the same buffer by using a dialysis membrane. The fraction in the dialysis membrane was applied to DEAE Bio-Gel A (product of Bio-Rad Laboratories) equilibrated with a 10 mM tris HCl buffer (pH 7.5) containing 2 mM calcium chloride to collect the protease active fraction not adsorbed to the ion-exchanger. This active fraction was applied further to "SP-Toyopearl 550W" (product of Tosoh Corp.) equilibrated with the same buffer, followed by elution with a 0 to 50 mM sodium chloride solution, whereby a protease active fraction was obtained. The resulting fraction was analyzed by SDS-PAGE electrophoresis to confirm that the protease was obtained as substantially uniform protein. The protein concentration was measured in

accordance with the method of Lowry, et al. (J. Biol. Chem. **193**, 265-275(1981)) by using bovine serum albumin (product of Bio-Rad Laboratories) as a standard.--

Please replace the paragraph bridging pages 30 and 31 with the following:

--In 2 mL of a 100 mM borate buffer (pH 10.5) containing 3% of aqueous hydrogen peroxide, a 50 [il] μL portion of each of the protease variants obtained by purification in Example 1 was added. The resulting mixture was allowed to stand at 30°C for 30 minutes. After addition of an adequate amount of catalase (product of Boehringer Manheim) to remove excess hydrogen peroxide, the residual protease activity was measured by the synthetic substrate assay. In FIG. 3, the residual activity after treatment with aqueous hydrogen peroxide is shown relative to the activity before treatment set at 100%.--

IN THE CLAIMS

--1. (Amended) An alkaline [protease] protease, wherein an amino acid residue at (a) position 84, (b) position 104, (c) position 256 or (d) position 369 of SEQ ID NO:1 or at a position corresponding thereto has been deleted or selected from:

at position (a): an arginine residue,

at position (b): a proline residue,

at position (c): an alanine, serine, glutamine, valine, leucine, asparagine, glutamic acid or aspartic acid residue, and

at position (d): an aspartic acid residue

2. (Amended) An alkaline [protease having] protease, comprising an amino acid sequence represented by SEQ ID NO:1 or having an amino acid sequence showing at least 60% homology therewith, wherein an amino acid residue at (a) position 84, (b) position 104,

(c) position 256 or (d) position 369 of SEQ ID NO:1 or at a position corresponding thereto has been deleted or selected from:

at position (a): an arginine residue,

at position (b): a proline residue,

at position (c): an alanine, serine, glutamine, valine, leucine, asparagine, glutamic acid or aspartic acid residue, and

at position (d): an aspartic acid residue.

3. (Amended) An alkaline [protease] protease, wherein an amino acid residue at (e) position 66 or 264, (f) position 57, each of 101 to 106, 136, 193 or 342, (g) position 46 or 205, (h) position 54, 119, 138, 148 or 195, (i) position 247, (j) position 124, (k) position 107 or (l) position 257 of SEQ ID NO:1, or at a position corresponding thereto has been deleted or selected from:

at position (e): a glutamine, aspartic acid, serine, glutamic acid, alanine, threonine, leucine, methionine, cysteine, valine, glycine or isoleucine residue

at position (f): a lysine, serine, glutamine, phenylalanine, valine, arginine, tyrosine, leucine, isoleucine, threonine, methionine, cysteine, tryptophan, aspartic acid, glutamic acid, histidine, proline or alanine residue,

at position (g): a tyrosine, tryptophan, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, lysine, glutamine, methionine or cysteine residue,

at position (h): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, histidine, serine, lysine, glutamine, methionine, glycine, aspartic acid, proline, arginine or cysteine residue,

at position (i): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, glutamine, methionine or cysteine residue,

at position (j): an alanine or lysine residue,

at position (k): a lysine, arginine, alanine or serine residue, and

at position (l): a valine or isoleucine residue.

4. (Amended) An alkaline [protease having] protease, comprising an amino acid sequence represented by SEQ ID NO:1 or having an amino acid sequence showing at least 60% homology therewith, wherein an amino acid residue at (e) position 66 or 264, (f) position 57, each of 101 to 106, 136, 193 or 342, (g) position 46 or 205, (h) position 54, 119, 138, 148 or 195, (i) position 247, (j) position 124, (k) position 107 or (l) position 257 has been deleted or selected from:

at position (e): a glutamine, aspartic acid, serine, glutamic acid, alanine, threonine, leucine, methionine, cysteine, valine, glycine or isoleucine residue

at position (f): a lysine, serine, glutamine, phenylalanine, valine, arginine, tyrosine, leucine, isoleucine, threonine, methionine, cysteine, tryptophan, aspartic acid, glutamic acid, histidine, proline or alanine residue,

at position (g): a tyrosine, tryptophan, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, lysine, glutamine, methionine or cysteine residue,

at position (h): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, histidine, serine, lysine, glutamine, methionine, glycine, aspartic acid, proline, arginine or cysteine residue,

at position (i): a tryptophan, phenylalanine, alanine, asparagine, glutamic acid, threonine, valine, leucine, isoleucine, histidine, serine, glutamine, methionine or cysteine residue,

at position (j): an alanine or lysine residue,

at position (k): a lysine, arginine, alanine or serine residue, and

at position (l): a valine or isoleucine residue.

5. (Amended) [An] The alkaline protease according to [claim 2 or 4] Claim 2, wherein the amino acid sequence represented by SEQ ID NO:1 or amino acid sequence showing at least 60% homology therewith is an amino acid sequence selected from [SEQ ID NOS: 2 to 7] a group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

6. (Amended) A gene encoding [an] the alkaline protease [as claimed in any one of claims 1 to 5] according to Claim 1.

7. (Amended) A recombinant vector comprising [a] the gene [as claimed in claim 6] according to Claim 6.

8. (Amended) A transformant comprising [a] the recombinant vector [as claimed in claim 7] according to Claim 7 .

9. (Amended) [A] The transformant according to [claim] Claim 8, wherein a microorganism is used as a host.

10. A detergent composition comprising [an] the alkaline protease [as claimed in any one of claims 1 to 5] according to Claim 1.--

--Claims 11-36 are new.--